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Ultrastructure of Subventral Gland Secretory Granules in Parasitic Juveniles of the Soybean Cyst Nematode, *Heterodera glycines*[1]

BURTON Y. ENDO[2]
U.S. Department of Agriculture, Agricultural Research Service, Plant Sciences Institute, Nematology Laboratory, Beltsville, Maryland 20705-2350

ABSTRACT: Secretory granules of the subventral esophageal glands of *Heterodera glycines* showed considerable changes in morphology soon after penetration and following initiation of syncytia in soybean roots. Within 3 hr after inoculation, the moderately dense secretory granules, usually found in subventral gland cells and their extensions, became electron-transparent except for small electron-dense residues within the secretory granule membranes. In samples taken 18 hr after inoculation and beyond, subventral gland extensions of parasitic second- and third-stage juveniles contained small, electron-dense secretory granules. The subventral glands were also characterized by the presence of moderate to very large flocculate secretion bodies within a dense matrix of rough endoplasmic reticulum, mitochondria, and Golgi apparatus. The activity of the Golgi apparatus was directly related to the formation and accumulation of condensing vesicles that appeared to merge with each other to form the larger secretory granules occurring in the subventral glands of parasitic stages of the nematode. The large flocculate secretion bodies were observed as early as 10 hr after inoculation and contrasted with the dense cytoplasm of the subventral glands observed 6 days after inoculation. The synthesis, assembly, accumulation, and transport of secretory granules within the subventral glands of the soybean cyst nematode appeared to change during parasitism.

KEY WORDS: *Heterodera glycines,* Nematoda, secretory granules, soybean cyst nematode, ultrastructure.

Subventral and dorsal glands are prominent features of the esophagus of tylenchid nematodes. Secretory granules formed in these glands are of major interest in understanding host–parasite interactions of plant-parasitic nematodes of major crop plants (Bird, 1968a, b, 1969; Rumpenhorst, 1984; Wyss et al., 1984; Hussey, 1989a; Endo, 1991). The extensions of the subventral glands in second-stage juveniles (J2) of the root-knot nematode, *Meloidogyne javanica,* accumulate secretory granules shortly before hatching and the granules change in morphology within 1–3 days after entry into the host. Meanwhile there was a 3-fold enlargement of the dorsal and subventral glands (Bird, 1967). These studies stimulated interest in esophageal gland structure–function relations in other species of endoparasitic nematodes. Changes in morphology of secretory granules in the dorsal esophageal gland occur between preparasitic and parasitic J2 stages of development in the cyst nematode, *Heterodera glycines.* Secretory granules in dorsal glands of parasitic J2 varied substantially in size and electron density from small moderately electron-dense secretory granules to large low-density secretory granules as feeding occurred (Endo, 1987). Previous work on *M. javanica* (Bird, 1967, 1975; Bird and Saurer, 1967) and in vivo observations of *Heterodera schachtii* (Wyss, 1992) emphasize that subventral glands play an active role in the parasitic behavior of these and related nematodes. This study emphasizes the changes in morphology of secretory granules of subventral esophageal glands and their sites of synthesis and modification during the infection of soybean by the soybean cyst nematode.

Materials and Methods

Preparasitic and parasitic stages of *H. glycines* in infected soybean (*Glycine max*) roots were prepared for electron microscopy by previously described procedures (Endo and Wergin, 1973; Wergin and Endo, 1976; Endo, 1978). Seedlings of susceptible and resistant cultivars were raised in vermiculite and inoculated with infective juveniles (J2) of the soybean cyst nematode. The J2 and nematode-infected root segments, sampled systematically from 3-hr through 8-day intervals after inoculation from several experiments, were fixed in buffered 3% glutaraldehyde (0.05 M phosphate buffer, pH 6.8) at 22°C for 1.5–4 hr; washed for 1 hr in 6 changes of the same buffer; postfixed in 2% osmium tetroxide in the same buffer for 2 hr; dehydrated in an acetone series; and infiltrated with a low-viscosity resin (Spurr, 1969). Silver-gray sections of selected nematodes and root tissues were cut on an ultramicrotome with a diamond knife and mounted on uncoated

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[2] Research Plant Pathologist.

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75 × 300-mesh copper grids. The sections were stained with uranyl acetate and lead citrate and viewed in a Philips 301 or 400T electron microscope operating at 60 kV with 20-μm objective aperture.

Results

In this study, the ultrastructure of the subventral glands of the preparasitic J2 (Fig. 1 and inset) provides a basis for comparison of changes that take place in these organs after host penetration and establishment of second- and third-stage juveniles (J2 and J3) as parasites in susceptible and resistant root tissues.

J2 preparasitic stage

The subventral gland cells of preparasitic J2 had distinct nuclei and nucleoli and numerous Golgi bodies, mitochondria, and rough endoplasmic reticulum (RER) (Fig. 1). Sections through the Golgi apparatus in subventral glands showed secretory granules that apparently originated from stacks of cisternae (Fig. 1 inset). The matrix of the secretory granules in the gland cell was moderately electron-dense and contained a particulate electron-dense region.

J2 parasitic stage

Within 3 hr after penetration, many of the secretory granules in the gland extensions became electron-transparent except for the small electron-dense cores (Fig. 2). Secretory granules in the central region of the gland cell appeared moderately electron-dense, while others near the nucleus had lower electron density (Figs. 3–5). Golgi bodies were often located near the nucleus of the subventral gland and adjacent to the nuclear membrane (Figs. 3–5). However, Golgi bodies can occur throughout the central body of the cell (Fig. 4). Within 5 hr after inoculation of the resistant cultivar Pickett, secretory granules and related Golgi within the subventral gland were quite extensive (Figs. 6–8). As in the *susceptible reaction*, secretory granules in the ampullae were partially depleted of their contents (Fig. 6). There was a wide range of secretory granule morphology and content (Fig. 7) of granules accumulated in anterior region of the gland cell.

At 18 hr after inoculation of susceptible or resistant cultivars, secretory granules in the subventral gland extensions of J2 were small and electron-dense and had fine particulate contents. The electron-dense secretory granules in the ampulla of a J2 feeding on an initial syncytial cell of the resistant cultivar Bedford (Figs. 9, 10) are similar to secretory granules in subventral gland extensions and ampullae of J2 within susceptible cultivars.

The subventral glands of a parasitic J2, 2 and 3 days after inoculation, contained gland cells with nuclei, large flocculent secretion bodies, and smaller secretory granules. The electron-dense granules in the gland cell were similar in morphology to the secretory granules observed near the valves in the ampullae of the gland extension (Figs. 11, 13). Compared to these small electron-dense granules, the large flocculent secretory bodies (FSBs) within the subventral gland cell were electron-translucent and appeared to be confined to the cell body (Fig. 12). Most nematodes at this stage of development were well established at feeding sites where the nematode stylet was inserted or had access to a syncytium induced by the nematode.

At 4 days after inoculation, a nematode near a transitional stage of development, usually just prior to molt, showed subventral gland valves in open positions (Fig. 14) filled with material similar in density to contents of the secretory granules. The gland cell contained large flocculate secretion bodies, Golgi apparatus, mitochondria, and RER (Fig. 15).

J3 parasitic stage

At 5 days after inoculation, the J3 stage was recognized by the presence of the molted J2 cuticle. A section through the nerve ring of a J3 showed accumulations of subventral and dorsal gland secretory granules within their respective gland extensions (Fig. 16). The subventral gland secretory granules were relatively small and markedly different from the larger, moderately electron-dense secretory granules of the dorsal glands.

At 6–8 days after inoculation, parasitic J3 contained secretory granules in various stages of formation. At 6 days after inoculation, the subventral glands had FSBs (Fig. 17). At 8 days after inoculation, open subventral gland valves (Fig. 19) were filled with electron-dense contents that indicated the active role of the subventral glands in producing secretory granules. Similar expanded valves were observed in a J2 during host penetration at 5 hr (Fig. 18) and at the beginning of molt of a J2, 4 days after inoculation (Fig. 14).

Discussion

Defining a role for the subventral esophageal glands and their products in plant-parasitic

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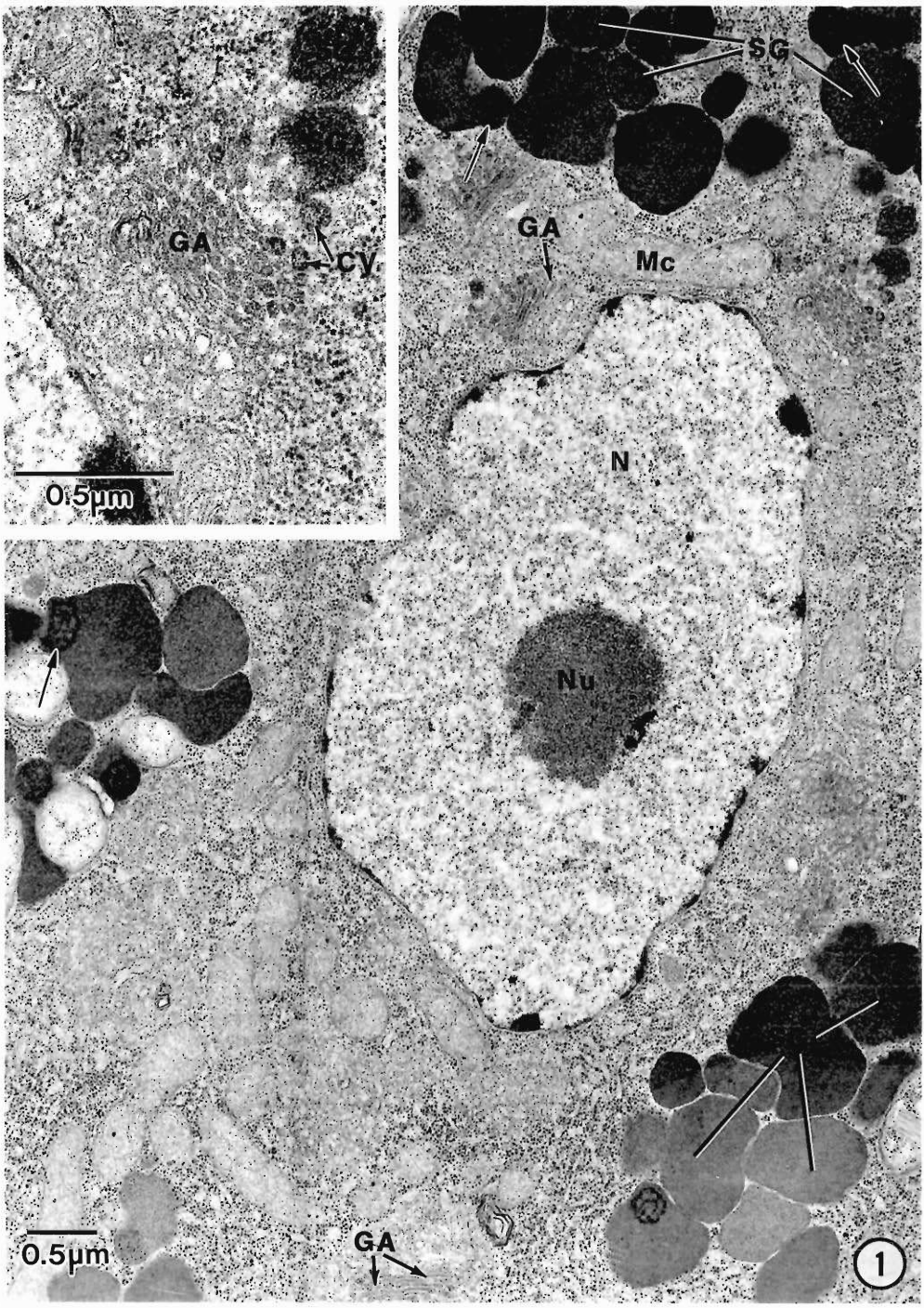

Figure 1. Longitudinal section through a subventral gland of a preparasitic J2 of *Heterodera glycines* showing electron-dense secretory granules (SG) and Golgi apparatus (GA) sites near the membrane of the gland nucleus (N) and other regions of the gland. Some secretory granules have electron-dense matrices (→). Mc, mitochondria; Nu, nucleolus. Inset shows enlargement of a Golgi apparatus (GA) and the transition from condensing vesicles (CV) to the larger secretory granules (SG).

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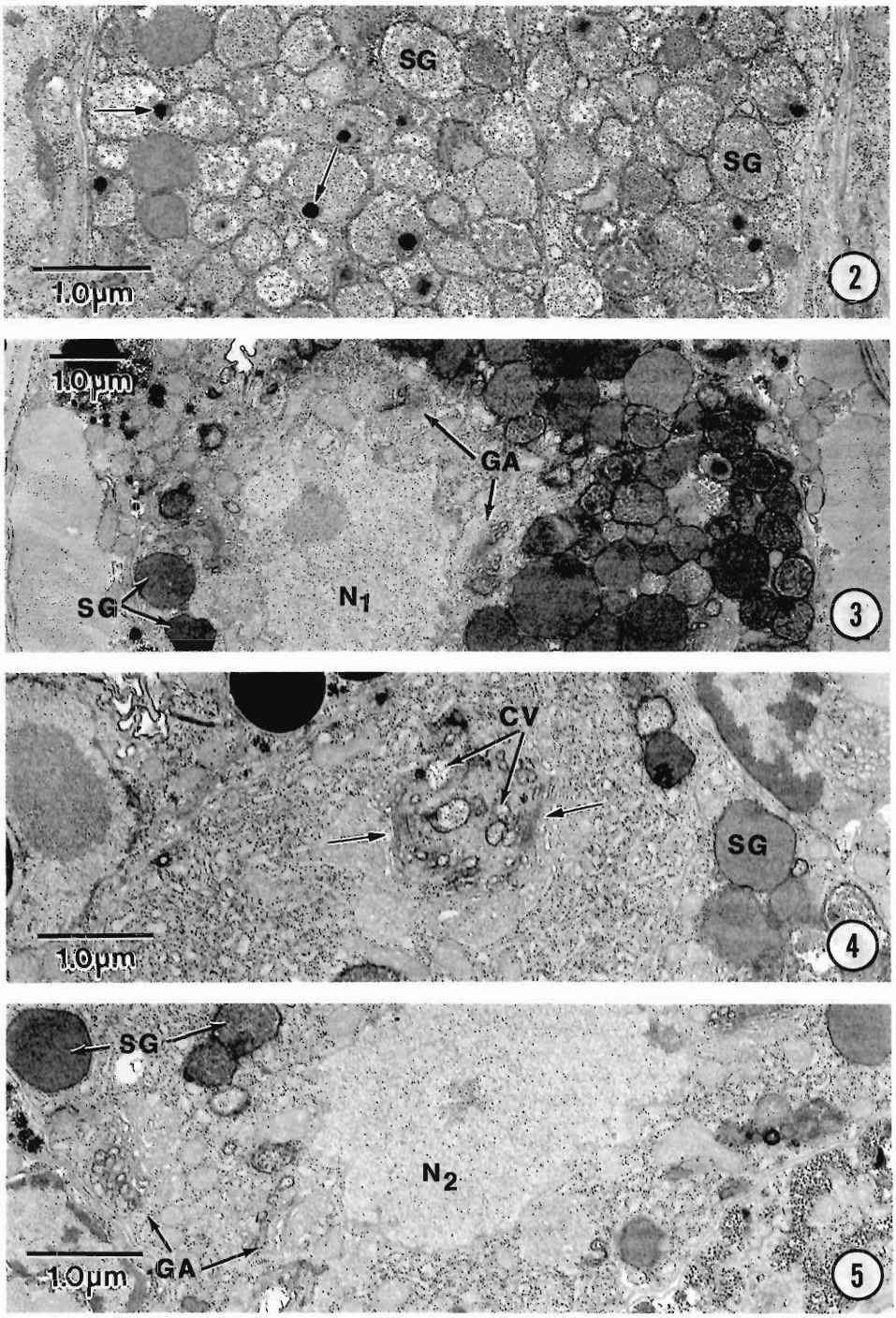

Figures 2–5. Longitudinal section through subventral gland cell extension of parasitic *Heterodera glycines* J2, 3 hr after inoculation of the susceptible soybean cultivar Lee. 2. Section near the lateroventral commissure of nerve fibers showing secretory granules (SG) of very low electron density, some with small electron-dense matrices (→). 3. Nucleus (N_1) in one of the 2 subventral gland cells surrounded by Golgi (GA) and numerous secretory granules (SG) with various levels of electron density. 4. Section showing a cluster of Golgi apparatus with trans-surface of cisternae stacks (→) facing each other. Condensing vesicles (CV) merge to form secretory granules (SG). 5. Nucleus (N_2) of the second subventral gland cell of same nematode surrounded with multiple sites of Golgi apparatus (GA) and secretory granules (SG) similar to those shown in Figure 3.

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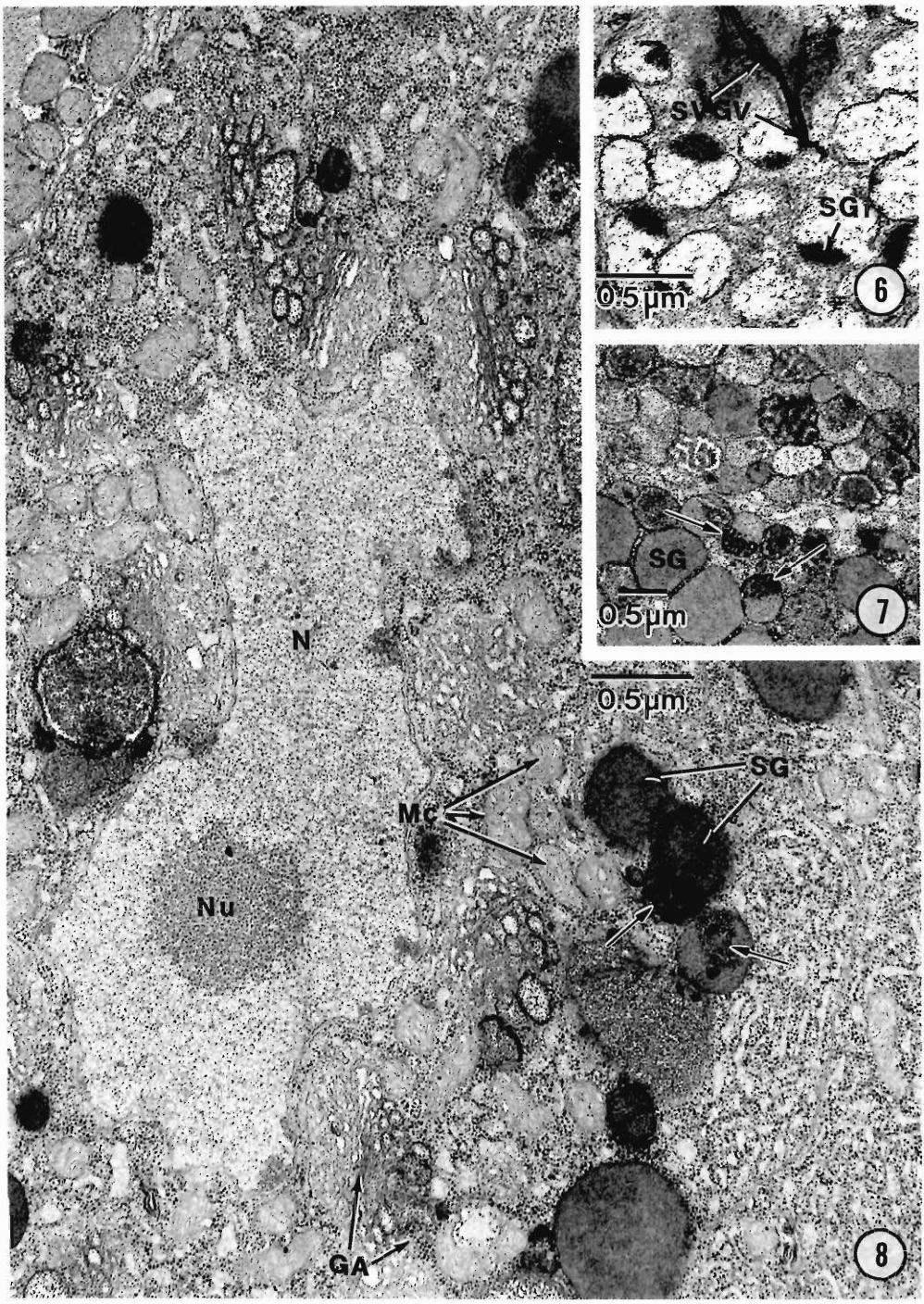

Figures 6–8. Transverse sections through sectors of the subventral gland of parasitic J2 of resistant cultivar Pickett, 5 hr after inoculation. 6. Secretory granules within ampulla are electron-translucent except for small residues of electron-dense material (SGr). SVGV, subventral gland valve. 7. Moderately dense secretory granules (SG) accumulated at anterior of gland body. Some granules contain electron-dense regions (→) within their granule matrices. 8. Section through the central region of the gland shows an enlarged nucleus (N) surrounded by Golgi (GA), secretory granules (SG), mitochondria (Mc), and a dense matrix of endoplasmic reticulum. →, electron-dense region; Nu, nucleolus.

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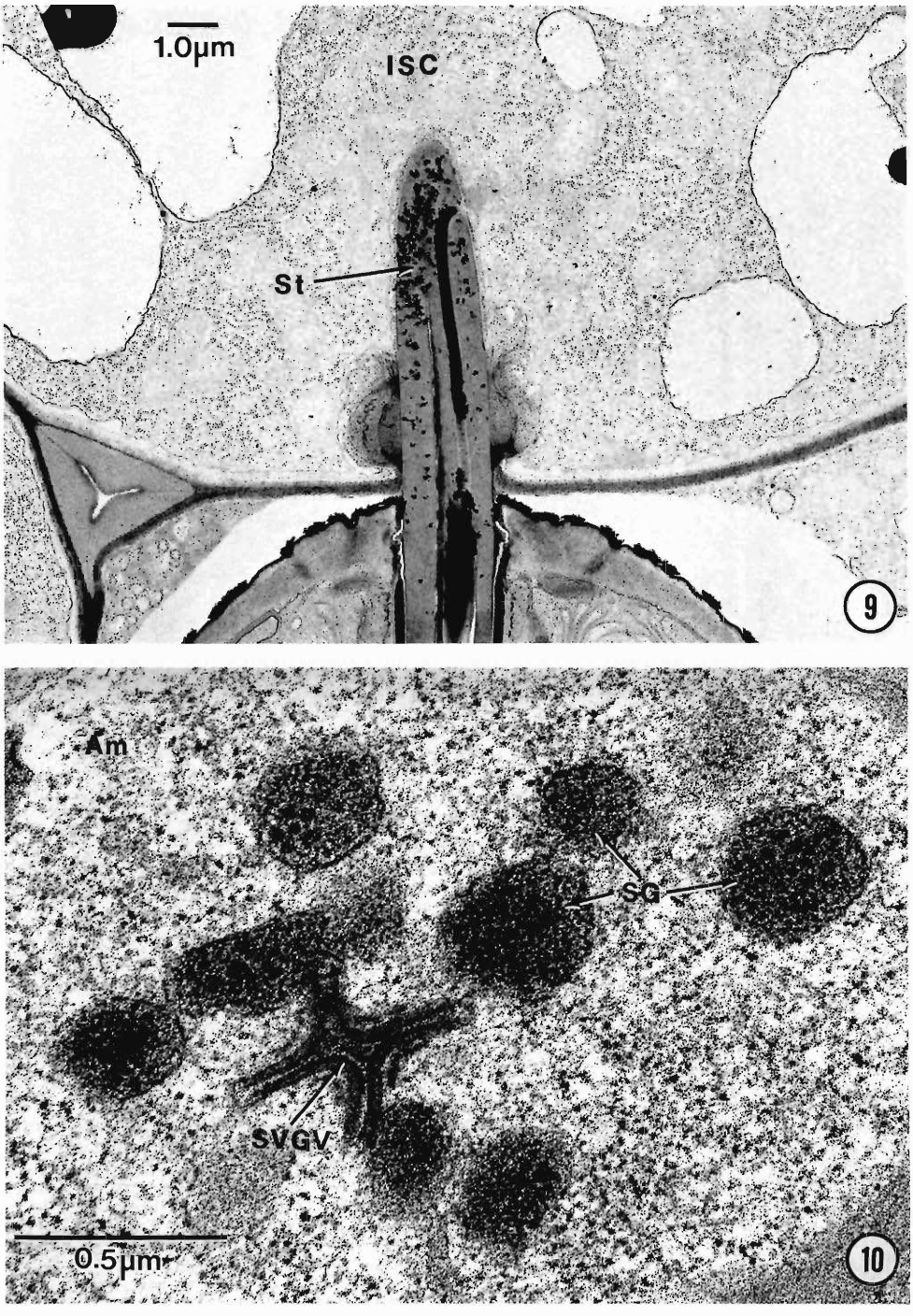

Figures 9, 10. Longitudinal sections of J2, 18 hr after inoculation at feeding site of resistant cultivar Bedford. 9. Lateral view of extended stylet (St) within the initial syncytial cell (ISC). 10. Electron-dense secretory granules (SG) within a subventral gland ampulla (Am). SVGV, subventral gland valve.

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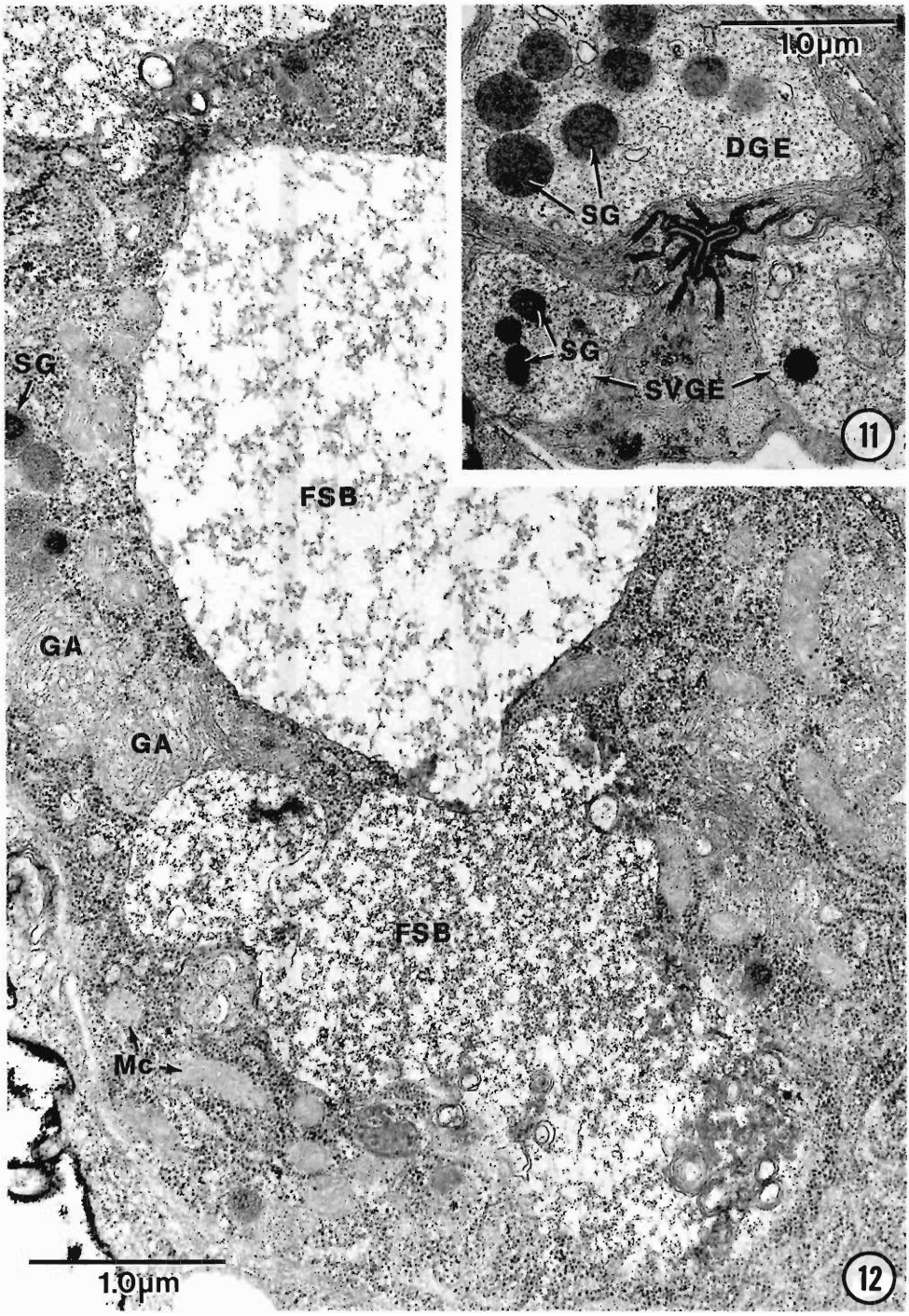

Figures 11, 12. Transverse sections through the esophagus of a specimen 2 days after inoculation in a parasitic phase of development and established at a feeding site with access to a syncytium and feeding tube. 11. Section through gland extensions anteriad from the nerve ring shows that the secretory granules (SG) of the subventral gland extensions (SVGE) are small with dense cores, while the secretory granules of the dorsal gland extension (DGE) are larger and moderately electron-dense. 12. Secretory granules (SG) in the gland extensions appear

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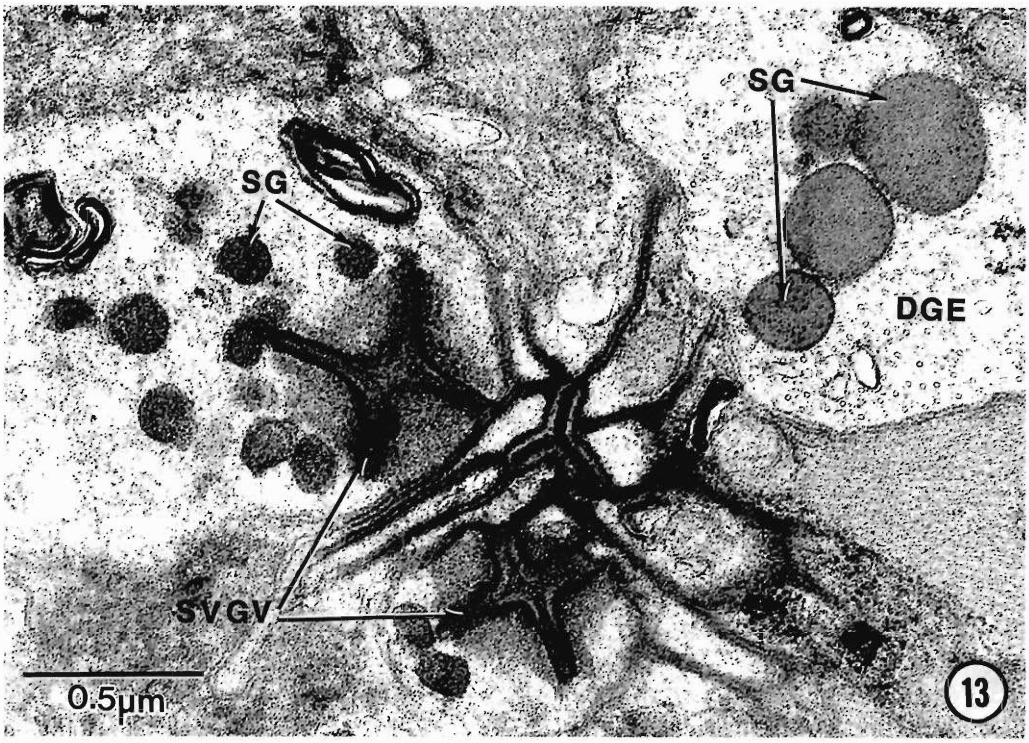

Figure 13. Transverse section of J2, 3 days after inoculation. Section shows closed subventral gland valves (SVGV) and accumulations of secretory granule (SG) contents near valves and within ampulla. DGE, dorsal gland extension.

nematodes has been based on light and electron microscope observations of the root-knot nematode, *M. javanica* (Bird, 1967, 1975). Histochemical studies by Bird and Saurer (1967) showed that the contents of the ducts of subventral glands changed their chemical composition within 2–3 days after nematode entry of the host. Bird (1967) proposed that dorsal and subventral glands play an important role in the transition from preparasitic to parasitic mode of nematode development. The subventral gland granules stained positive to periodic acid Schiff's reagent and the lumen of the esophagus became filled with material that resembled the internal contents of the granules. It was assumed the secretion(s) had an important role in the establishment of the nematode-induced giant cells (Bird, 1975). Marked chemical and morphological changes were induced by the host at the onset of parasitism (Bird, 1967). Subsequently, the subventral glands decreased in size and the dorsal gland enlarged. Stylet secretions apparently originating from secretory granules of the dorsal gland ampulla were thought to accelerate the development of the giant cells (Bird, 1968a). The change in size of the subventral gland granules reported by Bird (1975) has also been reported by Hussey and Mims (1990) in *Meloidogyne incognita*. The morphology and size of the secretory granules changed after the initiation of parasitism. An electron-dense zone developed in the periphery of the matrix of each granule that was often separated from the limiting membrane (Hussey and Mims, 1990). The electron-transparent core observed in the granules of preparasitic juveniles was absent in 7-day-old parasitic

←

similar to the secretory granules of Figure 11 accumulated near a Golgi apparatus (GA) within the central body of the gland cell. The cytoplasm contains numerous sites of Golgi apparatus. As in earlier infections, flocculent secretory bodies (FSB) occupy large regions of the subventral gland. Mc, mitochondria.

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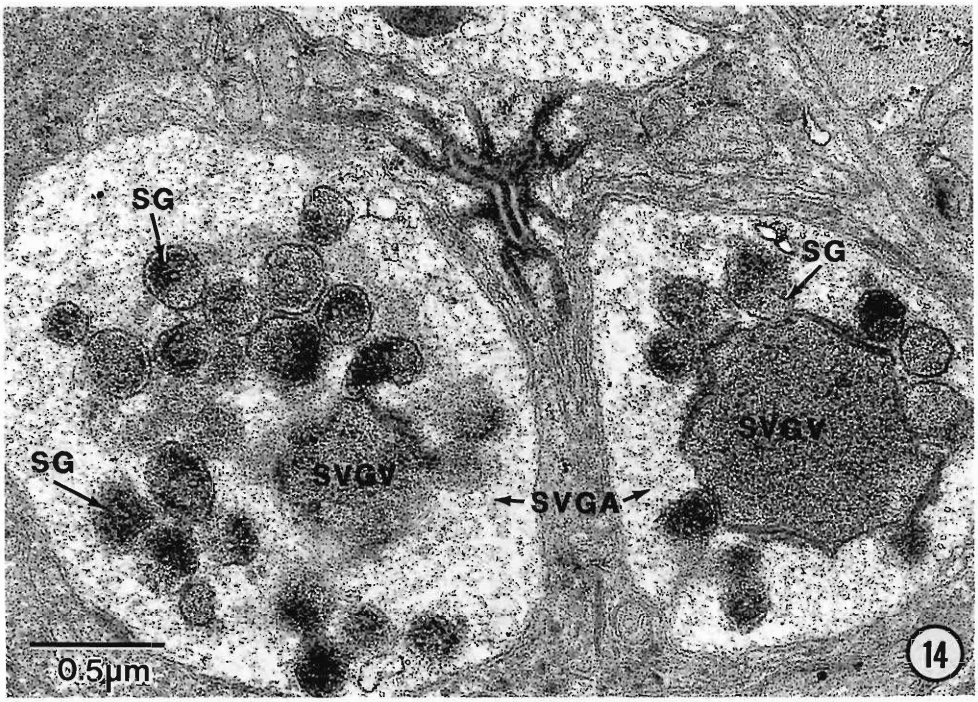

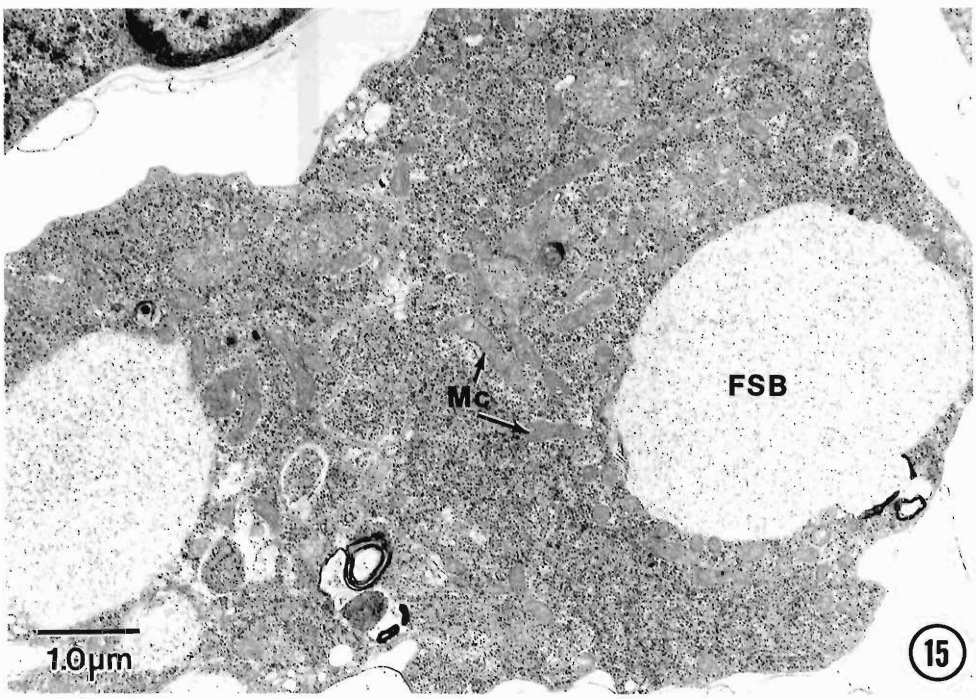

Figures 14, 15. Transverse sections through a J2 at 4 days after inoculation. 14. Section shows subventral gland valves (SVGV) in open positions containing electron-dense material and secretory granules (SG) with various levels of electron density. SVGA, subventral gland ampulla. 15. A subventral gland showing electron-translucent flocculent secretory bodies (FSB) within a dense matrix of cytoplasm. Mc, mitochondria.

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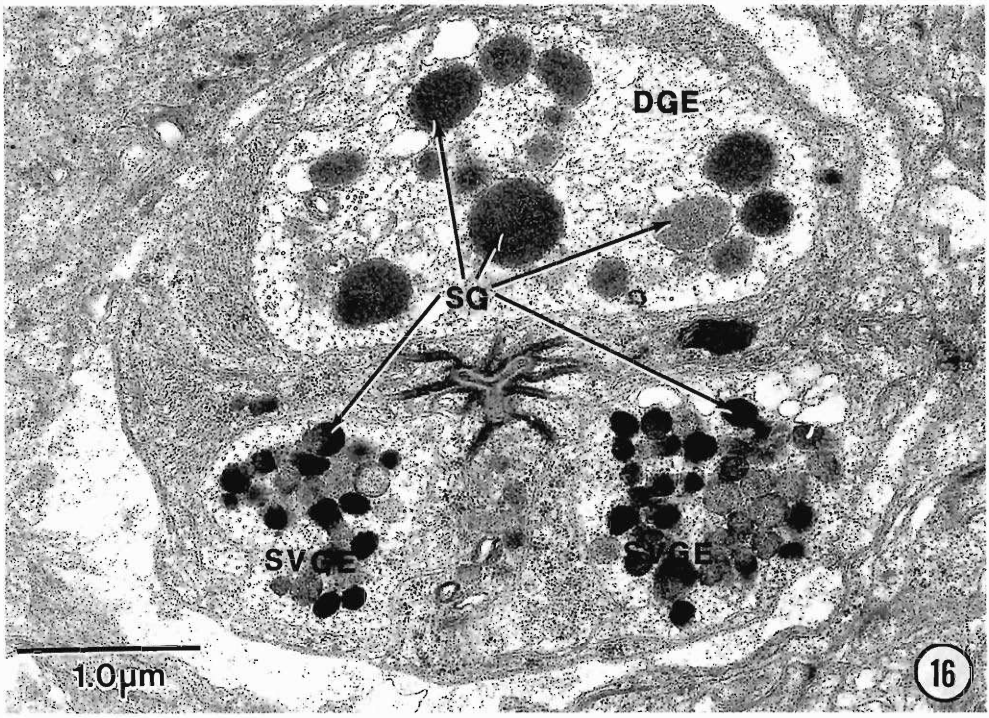

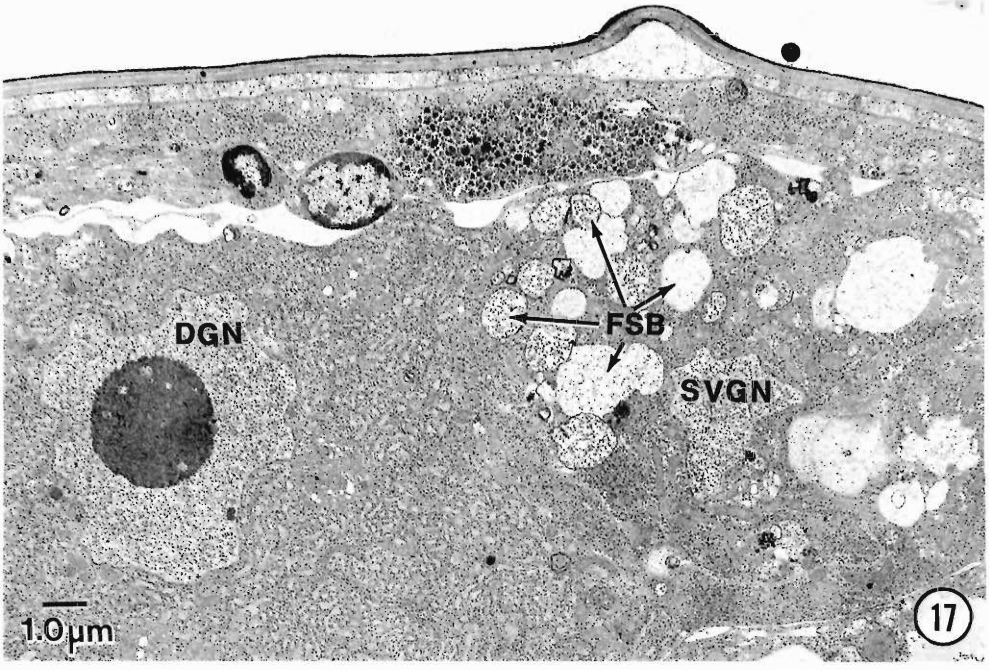

Figure 16. Transverse section through esophageal gland extensions and subventral cell of parasitic J3 at 5 days after inoculation. Gland extensions in nerve ring region showing contrast in secretory granule morphology of dorsal (DGE) and subventral gland extensions (SVGE). Secretory granules (SG) of subventral glands are considerably smaller than those of the dorsal gland and vary widely in electron density.

Figure 17. Longitudinal section through the dorsal and subventral glands of a J3 at 6 days after inoculation. Section shows nuclei of the dorsal (DGN) and of one of the subventral glands (SVGN). Moderately sized flocculent secretory bodies (FSB) with various levels of electron density are distributed throughout the subventral gland.

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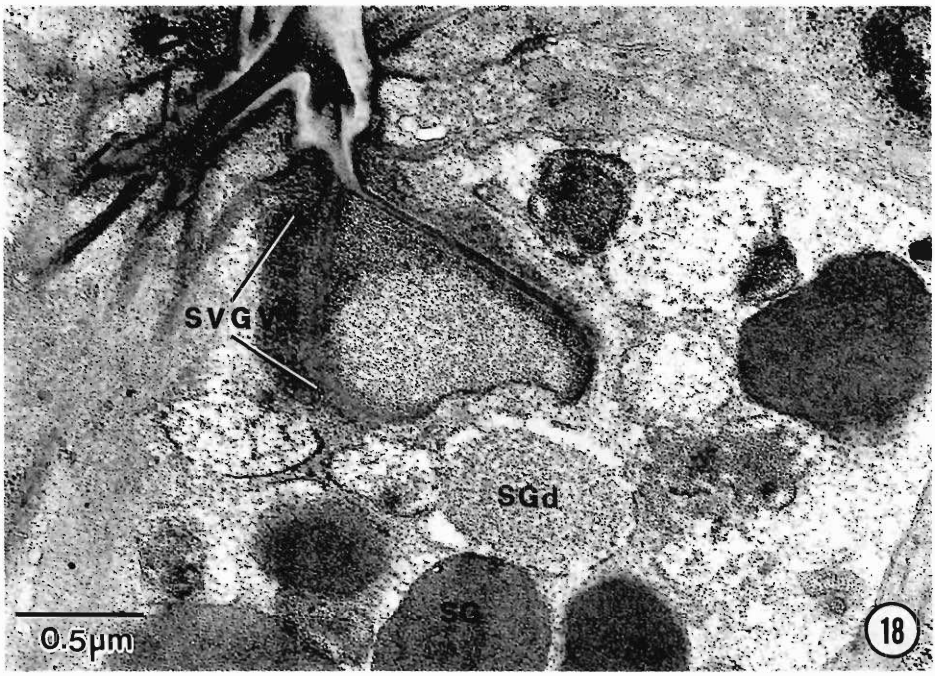

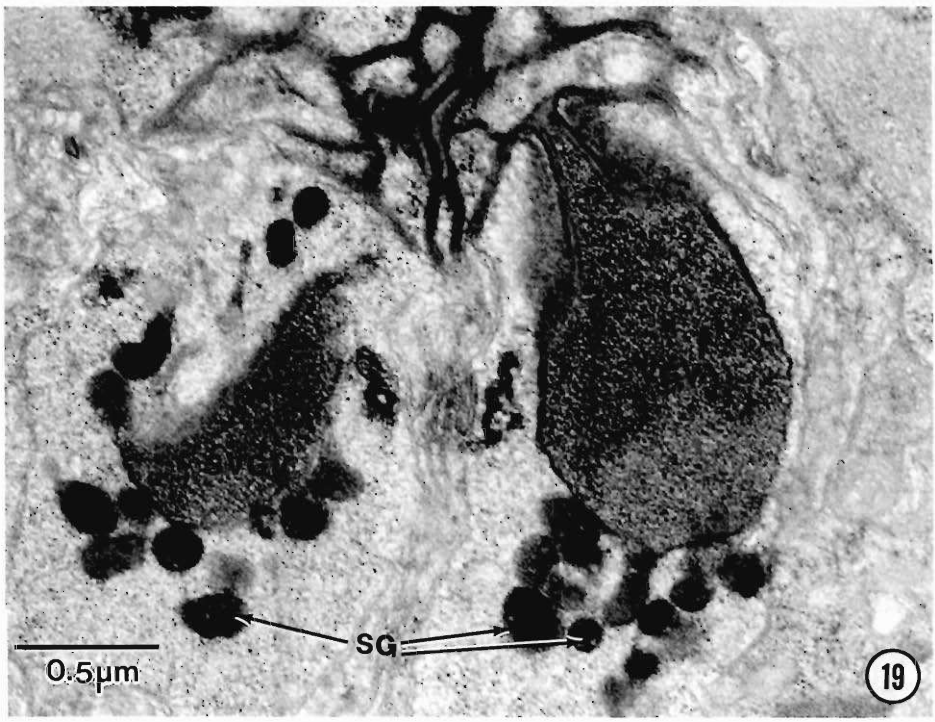

Figures 18, 19. Comparative sections of subventral gland valves of J2 at 5 hr and late J3 at 8 days after inoculation. 18. Longitudinal section of J2 during root penetration 5 hr after inoculation shows a secretory granule (SGd) with partially depleted contents adjacent to valve membrane. Electron-dense secretory granules (SG) in the ampullae are similar in electron density to the numerous granules in the gland extension. SVGV, subventral gland valve. 19. Open subventral gland valves (SVGV) of J3 filled with electron-dense material similar to the contents of adjacent secretory granules (SG) in the ampullae.

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J2 of *M. incognita*. Subventral gland secretory granules were about three-quarters the diameter of those in the preparasitic juveniles. These authors concluded that the subventral glands that were actively synthesizing secretory components as preparasitic J2 appeared to become inactive after the nematode established a feeding site. Existing secretory granules in the gland extensions in parasitic juveniles shrank and appeared to degenerate (Hussey and Mims, 1990). In the current study, many subventral secretory granules have spherical inclusions similar to those described in preparasitic *M. incognita* J2 by Hussey and Mims (1990). Current observations suggest that after the depletion of the rather large and peripherally electron-transparent secretion granules in gland extensions of the preparasitic J2, smaller electron-dense granules are synthesized in the gland cells of the parasitic J3. The very large FSBs found in the gland cells throughout the parasitic J2 and J3 stages are distinctive because of their electron transparency. Formation of these large flocculent bodies is difficult to determine; however, many appear to result from the merging of adjacent, moderately large, electron-transparent granules. The large flocculate secretory bodies and small secretory granules apparently arise from the activities of the Golgi apparatus that are scattered throughout the cytoplasm. Alternatively, the large FSBs may be formed directly from the endoplasmic reticulum. During the synthesis and enlargement of FSBs within the gland, smaller electron-dense secretion granules could be translocated anteriad toward the gland ampulla, as Wyss (1992) observed from in vivo studies with the closely related species *H. schachtii*.

The filling and release of secretory materials in the membranous valve of the subventral glands during in vivo observations of *H. schachtii* supports the concept that the subventral glands are actively involved in the parasitic stages of cyst nematode development (Wyss and Zunke, 1986). Similar in vivo studies of the entire life-cycle of *H. schachtii* (Wyss, 1992) showed all developmental stages with distinct feeding phases of food ingestion (I), stylet withdrawal and reinsertion (II), and salivation (III). In contrast to an earlier report based primarily on a single, ideally situated J2 (Wyss and Zunke, 1986), the filling and depletion of subventral gland valves was usually difficult to detect during phase II. However, the subventral glands were still active in producing secretory granules that accumulated in the gland ampullae of parasitic J2 and J3. The consistent and repetitive feeding cycles within the molting stages emphasize the vital role of the subventral gland and its secretory granules in the life-cycle of *H. schachtii*.

A similar functional relationship can be proposed for *H. glycines* and other related cyst-forming nematodes. The structure and function of the tetraradiate end-apparatus in the dorsal gland valve of the ectoparasite *Tylenchorhynchus dubius* (Anderson and Byers, 1975) and subsequent observations on the gland valves of *Ditylenchus dipsaci* (Shepherd and Clark, 1983) and *Heterodera glycines* (Baldwin et al., 1977; Endo, 1984) have been described. The valves of subventral glands in parasitic J2 and J3 of *H. glycines* frequently contained secretory components. Wyss and Zunke (1986) proposed that after secretory fluids accumulate in the subventral gland valves of *H. schachtii*, they are released to flow into the triradiate chamber of the metacorpus and then forced backward into the intestine through the triradiate lumen of the esophagus. Thus, both the dorsal gland and the subventral glands appear to be essential components of the parasitic stages of soybean cyst nematode during infection of soybean roots. Wyss (1992) also suggested that in *H. schachtii* J2 secretions from the subventral gland granules may be used to mobilize lipid reserves while the intestine is transformed into an absorptive organ during a preparation period. This nonfeeding period for *H. schachtii* starts after initial syncytial cell selection, which lasts several hours and is characterized by a marked decrease in density of the secretory granules in the ampullae and the extensions of the 2 subventral glands. The apparent release of secretions from secretory granules in the current study 3 hr after inoculation of *H. glycines* supports the assumption that most of the secretions may be used in a preparation period that transforms the preparasitic J2 into a parasitic J2.

Recent progress has been made in developing monoclonal antibodies for various components of esophageal glands of *H. glycines* (Atkinson et al., 1988; Atkinson and Harris, 1989), *Meloidogyne incognita* (Hussey, 1989b; Hussey et al., 1990), and various other species of *Meloidogyne* (Davis et al., 1991). This technology will provide ways to understand the mechanism of parasitism by localizing the site of nematode secretions in or associated with syncytia and giant cells induced by cyst and root-knot nematodes and will

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help to determine the function of secretory components synthesized in the subventral glands.

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